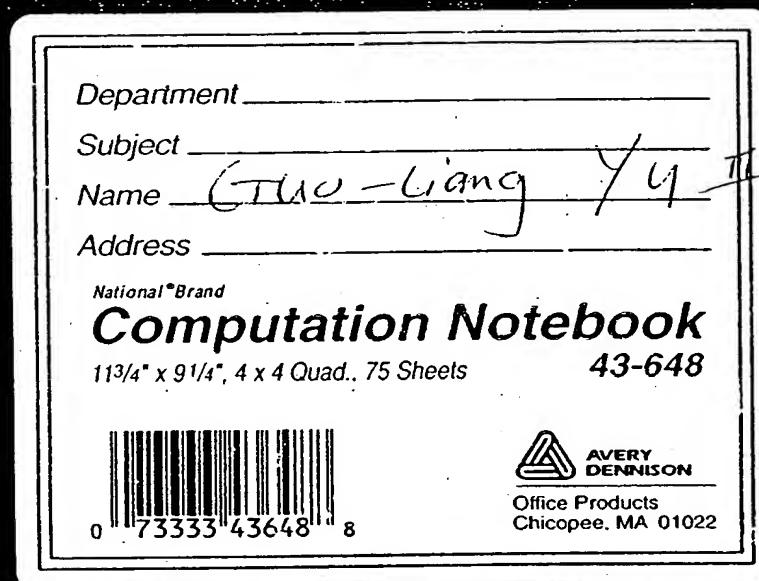


Steven M. Ruben
Appl. No. 10/662,429



Ruben EXHIBIT #63

Department _____

Subject _____

Name Guo-Liang Yu

Address _____

National® Brand

Computation Notebook

11 3/4" x 9 1/4", 4 x 4 Quad., 75 Sheets

43-648



0 73333 43648 8



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Ruben EXHIBIT 2063
Ruben v. Wiley et al.
Interference No. 105,077
RX 2063

1/23/96 Large scale plasmid prep for cDNA library

HBC to be used to make single stranded DNA

to test SAGE

transformation from an old stock of plasmid DNA

— obtain titer 1.5×10^7 cfu grow up to 1 L overnight

plasmid prep

sp. down, resuspend in 4 ml H2O
add some NaCl (100)
6 ml 3M KOAc

Spin down, ppt with isopropanol

CsCl₂ gradient 60K 0.6 hr

Concentration of DNA is low

2

1/24 proliferation assay for endothelial cells
EDAP

Cell lines Artery endothelial cell
 Venous endothelial cells
 Huvee

Seed 5×10^3 cells to each 96 well DMEM 10% FBS

5 hr incubation - let cell attach, remove medium

add samples as show below

	TNF α		EDAP		bFGF		EDAP		bFGF		EDAP	
	E2	E3										
A	1 ng	1 ng	1 ng	1 ng	10 ⁻⁹							
B	100 ng	100 ng	100 ng	100 ng	10 ⁻⁹							
C	10 ng											
D	1 ng											
E	10 ⁻⁹											
F	100 ng	100 ng	100 ng	100 ng	10 ng	10 ng	10 ng	10 ng	10 ng	10 ng	10 ng	10 ng
G	Control	10 ng	10 ng	10 ng	1 ng	1 ng	1 ng	1 ng	1 ng	1 ng	1 ng	1 ng
H		1 ng	1 ng	1 ng	0.1 ng	0.1 ng	0.1 ng	0.1 ng	0.1 ng	0.1 ng	0.1 ng	0.1 ng

40 hr after RTTS assay for A \bar{t} & VEG

add 3 H thymidine

incubate 16 hrs

wash once w/ PBS.

lyse cell Count

Certificate of Analysis

3

Protein Name: TNF gamma (EDAP)

Batch #: HG02704-E2

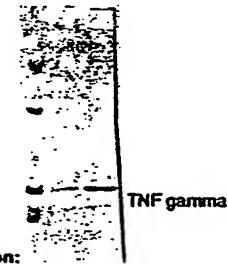
Date: 11/10/95

Expression System: E. Coli

Method of Purification:

1. Cell disruption and purification of inclusion body
- Wash pellet with TE, 1M, 2 M Urea, and
2. Extraction TNF γ with 4 and 8 M Urea
3. Buffer exchange (2XPBS) and refolding in PD-10
4. Removing endotoxin by affinity gel chromatography

Estimated Purity: > 80%



Protein Concentration:

Method A: 176 μ g/ml

Method B: 180 μ g/ml

Endotoxin level in sample: 0.17 EU/mg

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Sterile filtered: Yes No

Buffer composition: 2XPBS

Total amount of protein purified: 14 mg

Prepared by: Jian NI

Approved by: *Levin Jef*

1.5nd 53
14/30

Updated May 21, 1995

Certificate of Analysis

Protein Name: TNF gamma

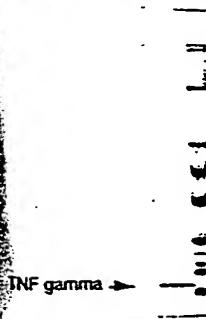
Batch #: HG02703-3E 43 4/5 - 5/6 Date: 5/26

Expression System: E. Coli

Method of Purification:

1. Cell disruption and purification of inclusion body
2. Solubilization of protein with 8 M urea
3. Sephadex S-200 size exclusion chromatography
4. Dialysis into PBS

Estimated Purity: > 70%



Protein Concentration: 80 μ g/ml

Endotoxin level in sample: 525 EU/ml

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Buffer composition: PBS

Endotoxin level in buffer: <10 EU/ml

Sterile filtered: Yes No

Prepared by: Jian NI

Approved by: *Levin Jef*

Updated May 21, 1995

Certificate of Analysis

Protein Name: TNF-delta (His-tag)
Clone ID#: HLTBT71

Batch #: HG10700-E1

Expression System: E. Coli

Molecular weight: 20.8 Kd

Method of Purification:
Nickel-chelate affinity chromatography column
Buffer exchange (2XPBS) and refolding in PD-10 column

Estimated Purity: 80%



Protein Concentration:

Method A: 200 μ g/ml

Method B: 160 μ g/ml

Endotoxin level in sample: 2.8 EU/ml

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Sterile filtered: Yes No

Buffer composition: 2XPBS

Biological activity
yes/not tested

Total amount of protein purified: 10 mg

Prepared by: Jian Ni

Approved by:

Updated September 15, 1995
1. μ -size exclusion chromatography
2. Dialysis into PBS

Estimated Purity: > 70%



TNF gamma \rightarrow

Protein Concentration: 80 μ g/ml

Endotoxin level in sample: 625 EU/ml

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Buffer composition: PBS

Endotoxin level in buffer: <10 EU/ml

Sterile filtered: Yes No

Prepared by: Jian Ni

Approved by: *Lei Lin*

Updated May 21, 1995

Certificate of Analysis

In Name: TNF gamma (EDAP)

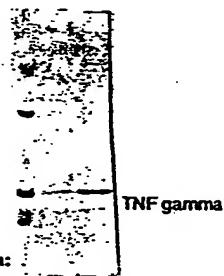
Batch #: HG02704-E2

Date: 11/10/95

Expression System: E. Coli

Method of Purification:
disruption and purification of inclusion body
pellet with TE, 1M, 2 M Urea, and
refold TNF γ with 4 and 8 M Urea
or exchange (2XPBS) and refolding in PD-10
column endotoxin by affinity gel chromatography

Estimated Purity: > 80%



Concentration:

Method A: 176 μ g/ml

Method B: 180 μ g/ml

Endotoxin level in sample: 0.17 EU/ml

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Certificate of Analysis

Protein Name: TNF-gamma (EDAP) (His-tag)
Clone ID#: HUVE091

Batch #: HG02702-E4

Date: 1/22/96

Expression System: E. Coli

Molecular weight: 20.1 Kd

Method of Purification:
Nickel-chelate affinity chromatography column
Buffer exchange (2XPBS) and refolding in PD-10 column

Estimated Purity: >80%



Protein Concentration:

Method A: 150 μ g/ml

Method B: 260 μ g/ml

Endotoxin level in sample: 3.1 EU/ml

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Sterile filtered: Yes No

Buffer composition: 2XPBS

Biological activity
yes/not tested

Total amount of protein purified: 10 mg

Prepared by: Jian Ni

Approved by:

Updated September 15, 1995

MTS array:

AT	1	2	3	4	5	6	7	8	9	10	11	12
A	0.019	0.033	0.070	0.033	0.016	0.013	0.016	0	0.012	0.281		
B	0.47	0.043	0.71	0.058	0.050	0.43	0.071	0.42	0.028	0.162		
C	0.039	0.071	0.73	0.040	0	0	0	0.078	0.025	0.028		
D	-0.051	0.011	0.020	0.014	0	0	0	0	-0.34	-0.029	0	
E	-0.027	0.038	0.043	0.010	-0.029	0.017	0.082	0.077	0.070	0.289		
F	-0.02	0.066	0.045	0.044	0.033	0.019	0.145	0.133	0.09	0.212		
G	0.015	0.04	0.028	0.02	0.007	0	0.086	0.091	0.1	0.059		
H	-0.01	0.061	0.072	0.092	0.039	0.001	0.042	0.013	0.041	0.017		

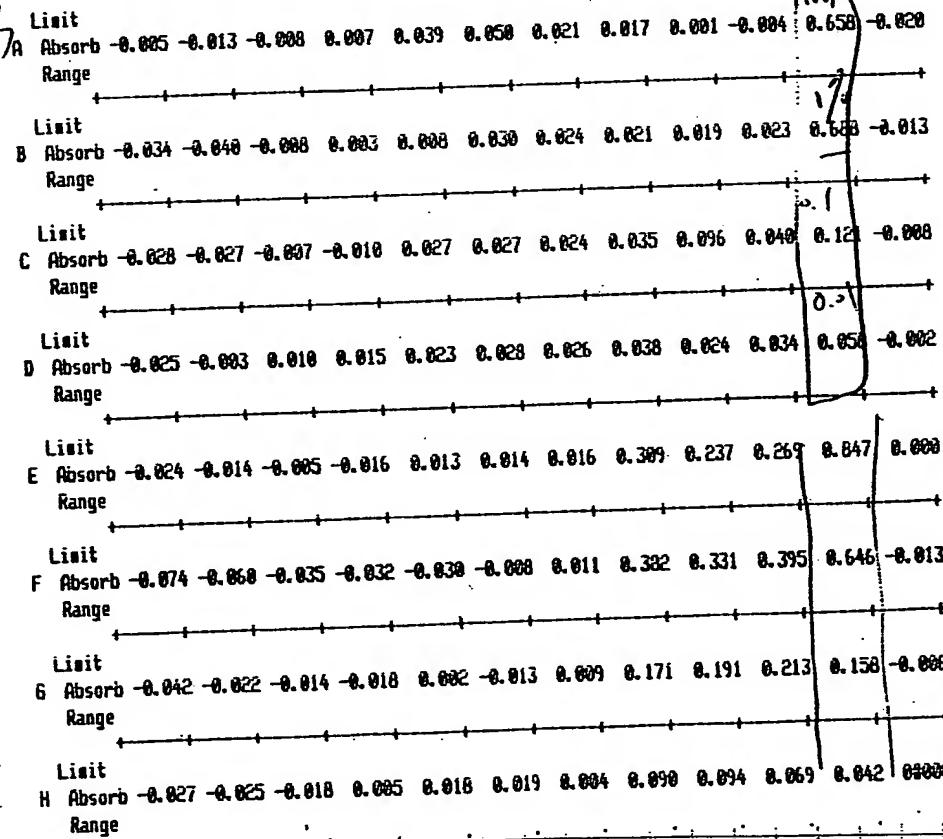
bf

G4

BFGF

FBS

1 2 3 4 5 6 7 8 9 10 11 12



V6

Fluvec

AE

USER: J ID:3H CPM PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#1 1 AOC:N GCF:N RCM:Y
 RCM-TIME: 0.50 INT:999.95
 CHANNEL 1-LL: 0 UL: 400 ZSIGMA: 0.50 BKG SUB: 0.00 BKG 2SIG
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:Q 1.000000
 HALF LIFE(DAYS):N

TUE 15 M

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG	HR	RCM#	81	243	1	340.00	19.83	1.00	99.82	59.0	32.02
1	163	1	233.00	12.57	1.00	1.19	62.0		82	244	1	62.00	22.09	1.00	100.86	59.0	46.70	
2	164	1	1532.00	5.08	1.00	2.42	61.0		83	245	1	70.00	23.90	1.00	102.09	54.0	46.85	
3	165	1	141.00	16.84	1.00	3.67	64.0		84	246	1	58.00	8.28	1.00	103.34	52.0	5.79	
4	166	1	301.00	11.33	1.00	4.90	66.0		85	247	1	55.00	25.20	1.00	104.57	51.0	37.26	
5	167	1	397.00	10.04	1.00	6.14	64.0		86	248	1	57.00	21.44	1.00	105.81	52.0	53.49	
6	168	1	223.00	13.39	1.00	7.38	64.0		87	249	1	175.00	15.12	1.00	107.04	59.0	19.57	
7	169	1	365.00	10.47	1.00	8.61	63.0		88	250	1	76.00	22.94	1.00	108.28	55.0	33.38	
8	170	1	336.00	10.91	1.00	9.85	64.0		89	251	1	59.00	26.04	1.00	109.52	53.0	32.55	
9	171	1	186.00	14.66	1.00	11.10	60.0		90	252	1	70.00	23.90	1.00	110.75	53.0	59.26	
10	172	1	116.00	18.57	1.00	12.34	63.0		91	253	1	50.00	29.28	1.00	111.98	52.0	59.88	
11	173	1	266.00	12.26	1.00	14.80	64.0		92	254	1	41.00	31.23	1.00	113.22	52.0	44.14	
12	174	1	633.00	7.94	1.00	16.04	63.0		93	255	1	14.00	30.15	1.00	114.45	51.0	23.34	
13	175	1	98.00	20.20	1.00	17.28	70.0		94	256	1	44.00	32.15	1.00	115.68	52.0	39.68	
14	176	1	233.00	13.10	1.00	18.52	63.0		95	257	1	147.00	16.50	1.00	116.71	54.0	44.00	
15	177	1	164.00	15.62	1.00	19.74	57.0		96	258	1	56.00	26.73	1.00	117.17	52.0	33.02	
16	178	1	36.00	26.73	1.00	20.97	60.0		97	259	1	52.00	27.74	1.00	119.40	50.0	36.20	
17	179	1	112.00	18.90	1.00	22.21	59.0		98	260	1	108.00	19.25	1.00	120.64	55.0	51.11	
18	180	1	101.00	19.90	1.00	23.46	61.0		99	261	1	163.00	15.67	1.00	121.87	52.0	29.10	
19	181	1	1932.00	4.55	1.00	24.67	61.0		100	262	1	53.00	27.47	1.00	123.11	51.0	23.20	
20	182	1	962.00	6.45	1.00	25.92	64.0		101	263	1	55.00	26.97	1.00	124.34	52.0	23.08	
21	183	1	357.00	10.59	1.00	27.16	67.0		102	264	1	138.00	17.03	1.00	125.59	55.0	50.81	
22	184	1	195.00	14.32	1.00	28.40	67.0		103	265	1	34.00	34.30	1.00	126.82	52.0	50.66	
23	185	1	687.00	7.63	1.00	29.63	63.0		104	266	1	42.00	30.86	1.00	128.05	52.0	35.53	
24	186	1	354.00	10.63	1.00	30.88	65.0		105	267	1	66.00	24.62	1.00	129.29	55.0	38.72	
25	187	1	177.00	15.03	1.00	32.13	66.0		106	268	1	136.00	16.01	1.00	130.54	58.0	63.75	
26	188	1	261.00	12.38	1.00	33.36	69.0		107	269	1	81.00	22.22	1.00	131.78	55.0	23.25	
27	189	1	248.00	12.70	1.00	34.59	64.0		108	270	1	119.00	18.33	1.00	133.02	57.0	50.75	
28	190	1	238.00	12.96	1.00	35.84	66.0		109	271	1	92.00	20.85	1.00	134.25	51.0	46.79	
29	191	1	477.00	9.16	1.00	37.08	67.0		110	272	1	168.00	15.43	1.00	135.50	52.0	52.65	
30	192	1	325.00	11.09	1.00	38.31	66.0		111	273	1	150.00	17.34	1.00	136.75	58.0	50.21	
31	193	1	337.00	10.59	1.00	39.56	66.0		112	274	1	183.00	14.59	1.00	138.00	59.0	61.70	
32	194	1	366.00	10.45	1.00	40.80	65.0		113	275	1	45.00	29.81	1.00	139.24	51.0	34.91	
33	195	1	331.00	10.68	1.00	42.03	65.0		114	276	1	61.00	25.61	1.00	140.47	54.0	39.45	
34	196	1	242.00	12.66	1.00	43.28	65.0		115	277	1	45.00	29.81	1.00	141.70	52.0	26.28	
35	197	1	323.00	11.09	1.00	44.53	64.0		116	278	1	134.00	16.12	1.00	142.95	60.0	61.37	
36	198	1	436.00	9.56	1.00	45.77	70.0		117	279	1	34.00	27.22	1.00	144.18	50.0	40.62	
37	199	1	473.00	9.20	1.00	47.00	66.0		118	280	1	29.00	37.14	1.00	145.41	52.0	28.05	
38	200	1	428.00	9.67	1.00	48.23	69.0		119	281	1	99.00	20.10	1.00	146.65	56.0	42.92	
39	201	1	297.00	11.61	1.00	49.48	68.0		120	282	1	37.00	32.88	1.00	147.88	60.0	27.07	
40	202	1	318.00	11.22	1.00	50.73	70.0		121	283	1	51.00	28.01	1.00	149.12	61.0	27.62	
41	203	1	348.00	10.72	1.00	51.97	65.0		122	284	1	63.00	25.20	1.00	150.35	57.0	23.21	
42	204	1	231.00	13.16	1.00	53.22	67.0		123	285	1	60.00	25.92	1.00	151.58	61.0	31.22	
43	205	1	394.00	10.08	1.00	54.47	70.0		124	286	1	67.00	24.43	1.00	152.82	55.0	24.78	
44	206	1	892.00	6.73	1.00	55.71	67.0		125	287	1	65.00	24.81	1.00	154.05	56.0	29.27	
45	207	1	115.00	18.63	1.00	56.96	68.0		126	288	1	47.00	29.17	1.00	155.27	57.0	16.35	
46	208	1	1239.00	3.68	1.00	58.20	67.0		127	289	1	45.00	29.81	1.00	156.51	61.0	18.46	
47	209	1	533.00	8.50	1.00	59.45	71.0		128	290	1	59.00	26.04	1.00	157.73	56.0	13.03	
48	210	1	244.00	12.80	1.00	60.69	67.0		129	291	1	60.00	25.82	1.00	158.97	56.0	48.20	
49	211	1	266.00	12.26	1.00	61.92	69.0		130	292	1	115.00	19.65	1.00	167.62	59.0	60.08	
50	212	1	433.00	9.59	1.00	63.16	64.0		131	293	1	102.00	19.80	1.00	168.86	61.0	58.56	
51	213	1	289.00	11.76	1.00	64.40	69.0		132	294	1	40.00	31.62	1.00	170.10	61.0	14.29	
52	214	1	197.00	14.25	1.00	65.74	67.0		133	295	1	43.00	30.50	1.00	171.33	59.0	20.39	
53	215	1	245.00	12.83	1.00	66.99	67.0		134	296	1	34.00	34.30	1.00	172.56	62.0	28.12	
54	216	1	333.00	10.96	1.00	68.23	68.0		135	297	1	82.00	22.09	1.00	173.79	56.0	31.15	
55	217	1	143.00	16.72	1.00	69.46	67.0		136	298	1	56.00	28.28	1.00	175.03	54.0	39.19	
56	218	1	183.00	14.78	1.00	70.71	67.0		137	299	1	73.00	23.57	1.00	176.27	55.0	37.92	
57	219	1	6583.00	2.47	1.00	71.94	64.0		138	300	1	69.00	24.08	1.00	177.49	54.0	23.80	
58	220	1	907.00	6.64	1.00	73.18	64.0		139	301	1	93.00	20.74	1.00	178.73	60.0	41.00	
59	221	1	1470.00	9.23	1.00	74.42	64.0		140	302	1	47.00	29.17	1.00	179.97	55.0	31.18	
60	222	1	335.00	10.93	1.00	75.65	64.0		141	303	1	44.00	30.15	1.00	181.20	55.0	42.84	
61	223	1	303.00	11.49	1.00	76.89	64.0		142	304	1	57.00	26.49	1.00	182.43	34.0	39.87	
62	224	1	230.00	12.65	1.00	84.35	66.0		143	305	1	56.00	33.33	1.00	183.67	35.0	44.03	
63	225	1	267.00	12.24	1.00	77.13	61.0		144	306	1	57.00	26.49	1.00	184.89	58.0	14.23	
64	226	1	307.00	11.41	1.00	78.36	66.0		145	307	1	38.00	32.44	1.00	186.12	62.0	20.38	
65	227	1	369.00	10.41	1.00	79.61	65.0		146	308	1	43.00	29.81	1.00	187.35	61.0	19.47	
66	228	1	404.00	9.75	1.00	80.85	68.0		147	309	1	52.00	15.74	1.00	188.60	60.0	39.16	
67	229	1	237.00	12.99	1.00	82.07	63.0		148	310</td								

3/8/96 induction of recombinant protein

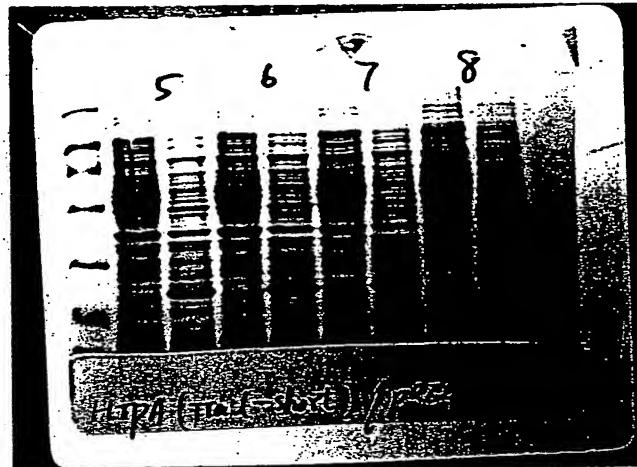
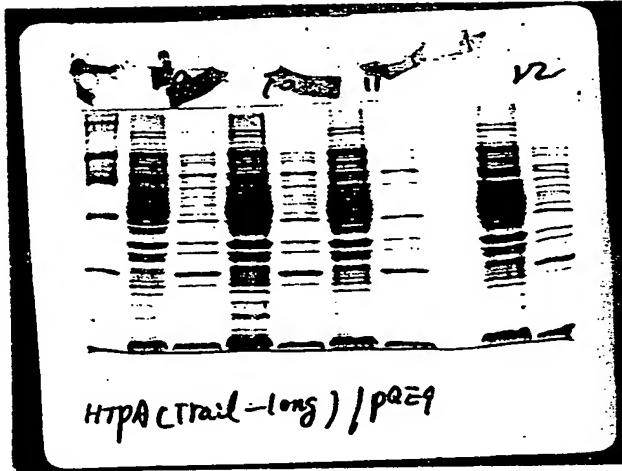


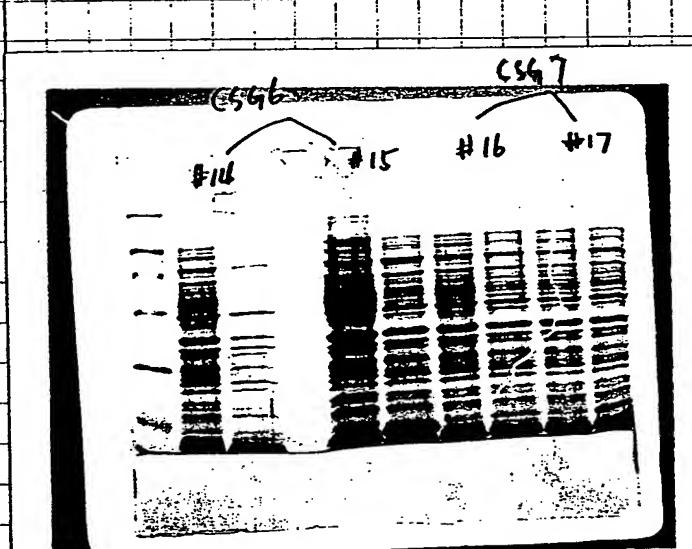
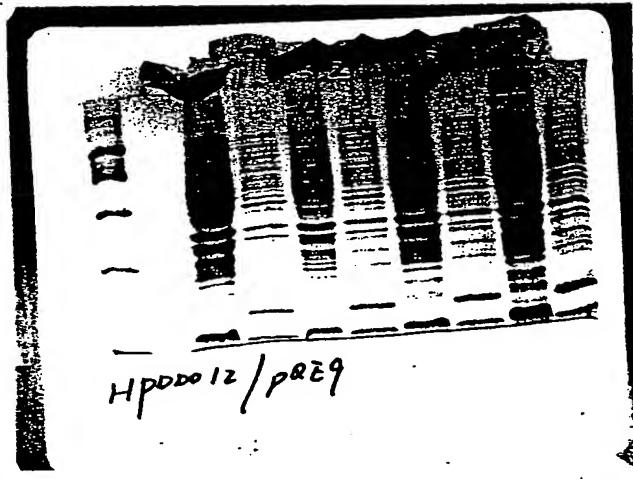
Figure 1. Nucleotide and Predicted Amino Acid Sequence of the TRAIL cDNA

The cDNA sequence of the human TRAIL gene is shown. Numbers at the right indicate the nucleotide number (top) and amino acid number (bottom). Amino acids comprising the predicted transmembrane region are underlined. Underlined nucleotides near the 3' end form a consensus polyadenylation signal.

BamH1 His-tagged

7

Tnf α expression in pOE β 9 His tagged

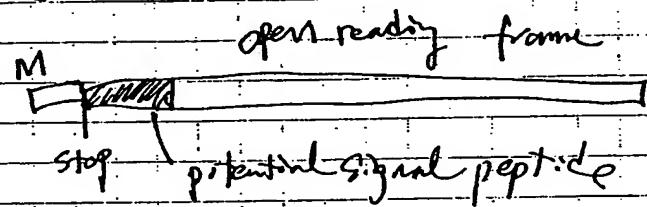


3/12/96 TNF Receptor like HTTBNG1

Now called Death Domain containing Protektor DD CR

Full length clone HLM17058 contained a stop codon

after methionine

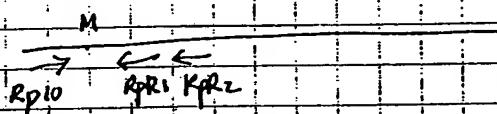


The stop codon is read with many rounds of sequencing

- ① To get new clones
- ② PCR 5' seq of start sequence

Oligo HTTBNG1Rp10 made:

CCCC TCCGAAG CCTG GTGT



use Rpl0 and Rpr2 to PCR libraries. Sequence the product with Rpr1

3/3/96 Expression vectors:

① EDAP $\Delta 2$ 5' CGCCATGGCTTACCAAGAACGA NcoI
 $\delta 48$

② EDAP $\Delta 3$ 5' CGCCCATGGACTATAACAA ~~SAAATTC~~ NcoI
 $\delta 54$

both clone into pQE60 3' will use EDAP HindIII

③ AIM2 $\Delta 1$ 5' CGCCATGGCCAACTCCAGCTTGACC NcoI

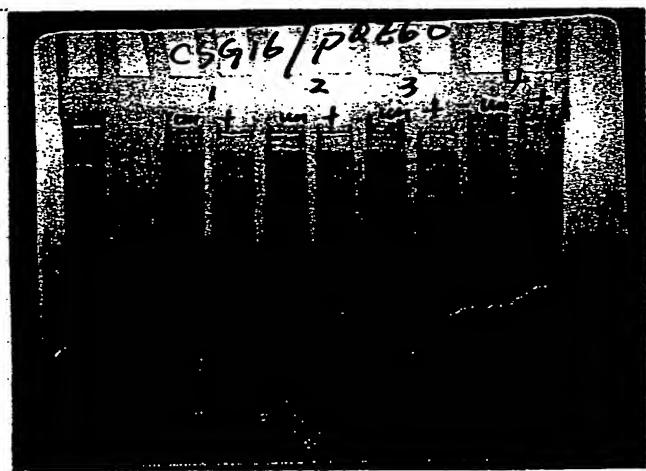
④ AIM2 $\Delta 2$ 5' CGCCATGGTCAACC ~~CGCCTG~~ CCT ~~GA~~ NcoI

both clone into pQE60 3' HindIII

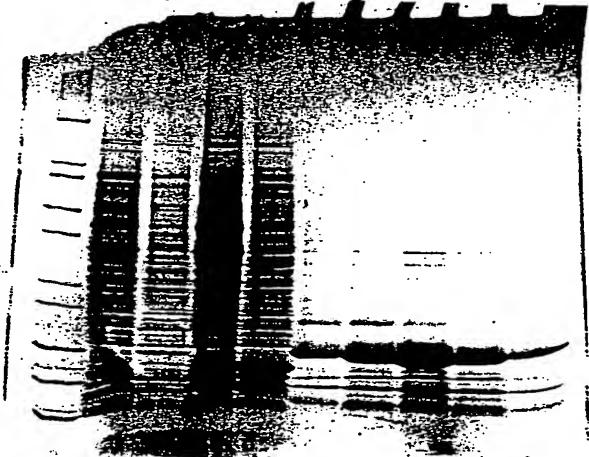
SUPERVISOR
DATE

REVIEWER
DATE

10

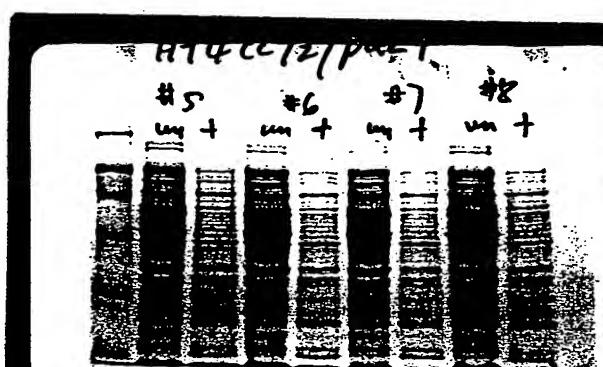


3/15/96

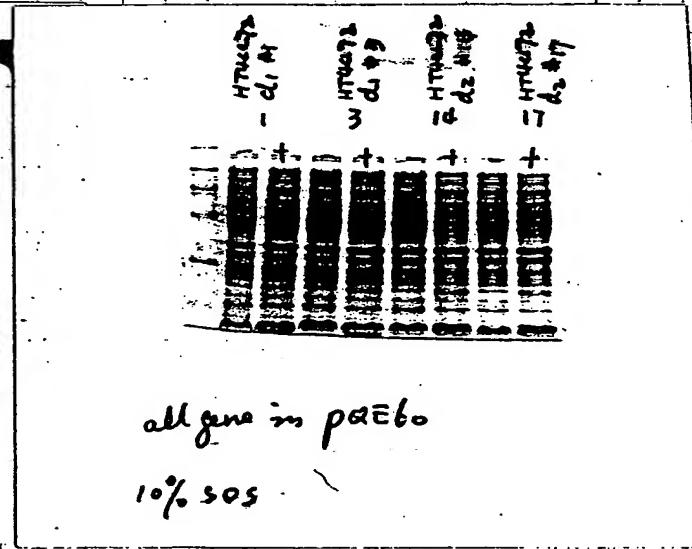


CSG

CSG

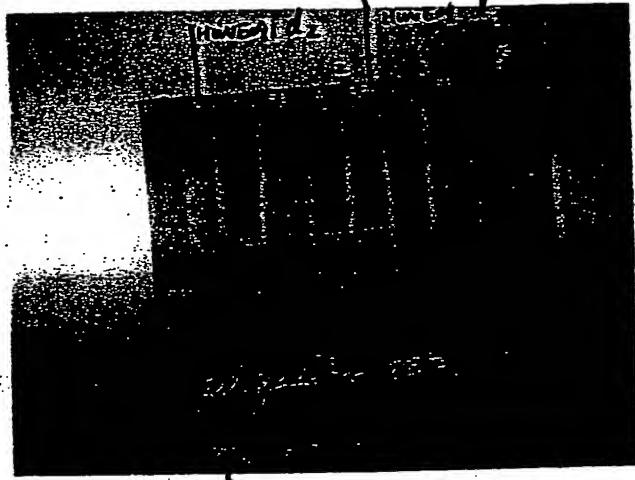


3/15/96



all gene in pQE60

10% SOS



EDAP Expression vector position

781 ATATGAGACGCTTTTAAGCAAAGTCTACAGTTCCAAATGAGAAAATTAACTCTCTTC
 M R R F L S K V Y S F P M R K L I L F L
 PQE9, PQE70, CHO
 841 TTGTCCTTCAGTTGTGAGACAAACTCCCACACAGCACTTTAAAATCAGTTCCCAGCTC
 V F P V Y R Q T P T Q H F K N Q F P A L
 IL6 PQE60, PA2-GP
 901 TGCACCTGGAAACATGAACTAGGCCTGGCCTTACCAAGAACCGAATGACTATACCAACA
 H W E H E L G L A F T K N R M M N Y T N K
 961 AATTCCCTGCTGATCCCAGAGTCGGGAGACTACTTCATTTACTCCAGGTACATTCCGTG
 F L L I P E S G D Y F I Y S Q V T F R G
 1021 GGATGACCTCTGAGTGCAGTGAATCAGACAGCAGGGGACCAAACAAGGCCAGACTCCA
 M T S E C S E I R Q A G R P N K P D S I
 1081 TCACTGTGGTCATCACCAAGGTAAACAGACAGCTACCCCTGAGCCAACCCAGCTCCTCATGG
 T V V I T K V T D S Y P E P T Q L L M G
 1141 GGACCAAGTCTGTATGCGAAGTAGGTAGCAACTGGTCCAGCCCATCTACCTCGGAGCCA
 T K S V C E V G S N W F Q P I Y L G A M
 1201 TGTTCTCCCTTGCAAGAAGGGGACAAGCTAATGGTGAACGTCAGTGACATCTCTTGGTGG
 F S L Q E G D K L M V N V S D I S L V D
 1261 ATTACACAAAAGAAGATAAAACCTCTTGGAGCCCTCTACTATAGGAGGAGAGCAAAT
 Y T K E D K T F F G A F L L *

δ38 d1

448 d2

454 d3

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